The influence of nitrogen and phosphorus levels on the phytotoxicity of phenolic compounds

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The interaction between the phytotoxicity of certain phenolic compounds and deprivation of certain nutrients was investigated by means of a factorial experiment. Two concentrations of *p*-coumaric acid and two of vanillic acid were added to nutrient solutions containing various quantities of nitrogen and phosphorus, and these solutions were tested on barley plants in sand culture. Phenolic toxicity appears to depend intimately on nutrient concentrations; the phenolic acids were uniformly and significantly inhibitory only at low nutrient concentrations. Allelopathy with phenolics as chemical inhibitors seems most likely to occur in nutrient-poor soils.

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L'interaction entre la phytotoxicité de certains composés phénoliques et la carence en certains éléments nutritifs a été étudiée par une expérience factorielle. Deux concentrations d'acide *p*-coumarique et deux concentrations d'acide vanillique ont été ajoutées à des solutions nutritives contenant diverses quantités d'azote et de phosphore et ces solutions ont été testées sur de l'orge cultivé dans du sable. La toxicité phénolique semble dépendre étroitement de la concentration des éléments nutritifs; les acides phénoliques produisent une inhibition uniforme et significative seulement en présence de faibles concentrations d'élements nutritifs. Une allélopathie due à des substances phénoliques se produirait surtout dans les sols pauvres en éléments nutritifs.

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Introduction

Despite a great volume of research on allelopathy, it remains unclear whether chemical interactions among plants are widespread and important in natural and agricultural plant communities (Harper 1977). Although there is evidence suggesting that many species from a variety of ecosystems are allelopathic (Rice 1974), some of the methodology of this field of plant ecology is open to question (Harper 1977; Newman 1978; Stowe 1979). The search for irrefutable cases of allelopathy would clearly proceed more rapidly with the knowledge of which plant communities are most likely to exhibit chemical inhibition of one plant by another. It is therefore important to understand the possible mechanisms of allelopathy and the conditions under which plants are most susceptible to phytotoxins.

Glass (1972, 1974) has found that phenolic compounds, the most commonly identified phytotoxins produced by higher plants (Rice 1974), inhibit the absorption of phosphate and potassium by excised roots in solution. These results may explain the finding of Schreiner and Skinner (1912) that inhibition of growth by phenolic compounds could be modified by adding certain nutrients to the water culture. Glass (1976) has also shown that a mixture of four phenolic compounds, in the concentrations Whitehead (1964) found in soil beneath a stand of bracken, was inhibitory in water culture, but only slightly inhibitory if nutrients were present in the solution.

In this study the interaction between phenolic toxicity and nutrient deprivation was further investigated by a sand-culture experiment, in an effort to determine whether certain phenolics are rendered less inhibitory by the presence of certain nutrients.

Methods

Barley (*Hordeum vulgare* L.) was chosen for this experiment because of its rapid and uniform growth, and because excised roots of barley were known to be influenced in their phosphorus and potassium uptake by certain phenolic compounds (Glass 1972, 1974). Seeds of barley (C. V. Beacon) were planted in quartz sand in plastic pots of 10 cm diameter. Ten seeds were planted in each pot at a uniform depth of 1 cm. The pots were assigned to one of seven nutrient treatments and one of five phenolic treatments, and were placed in a homogeneous portion of a greenhouse. They were watered with distilled water for 8 days; on the 9th day they were thinned to three uniform plants per pot and the nutrient treatments were begun.

The nutrient solutions followed the example of Bradshaw *et al.* (1958), Bradshaw *et al.* (1960), and Bradshaw *et al.* (1964). There were three low-phosphorus solutions, containing 1, 3, and 9 ppm phosphorus, and three low-nitrogen solutions, containing

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9, 27, and 81 ppm nitrogen. The complete nutrient solution contained 27 ppm phosphorus and 243 ppm nitrogen. All other nutrient concentrations were held constant, and these concentrations are listed in Bradshaw *et al.* (1958). Of course it is chemically impossible to vary the concentration of one ion in a nutrient solution without also varying the concentration of some other ions. As the phosphate concentration was reduced, so also was the concentration of sodium; and as the nitrate concentration was reduced, the concentration of sodium was reduced and that of chloride was increased, as described in Bradshaw *et al.* (1964). The plants were watered with these nutrient solutions once per day, 150 mL per pot. In order to prevent the accumulation of nutrients to potentially toxic levels, the pots were flushed once per week with 400 mL of distilled water.

The phenolic acid treatments were begun on the 11th day. There were five phenolic acid treatments, and each was given for each of the nutrient treatments (factorial design). *P*-coumaric acid was added to the nutrient solution to give concentrations of 5 ppm (3.05×10^{-5} *M*) and 10 ppm (6.09×10^{-5} *M*), and vanillic acid was added to give concentrations of 25 ppm (14.87×10^{-5} *M*) and 50 ppm (29.73×10^{-5} *M*); the control contained no phenolics. These particular phenolic compounds were chosen because they have often been found, not only in extracts of plant material (Börner 1960; Grümmer 1961; Guenzi and McCalla 1966; Abdul-Wahab and Rice 1967; del Moral and Muller 1970), but also in natural and agricultural soils (Whitehead 1964; Wang *et al.* 1967; Chou and Muller 1972; Lodhi 1978; Gant and Clebsch 1979). The concentrations were designed to give moderate but significant inhibition based on the investigations of Schreiner and Skinner (1912) and Wang *et al.* (1967).

Algae grew on the surface of the sand shortly after the addition of nutrients, a problem noted also by Bradshaw *et al.* (1960). A 1-cm layer of washed black gravel was added to the pots on the 15th day, and this modification arrested algal growth completely.

Forty-five days after planting, the plants were harvested, washed free of sand, separated into roots and shoots, dried in an oven at 80°C, and weighed. From the weight of roots and shoots, the total dry weight and the ratio of root dry weight to shoot dry weight (hereafter called the root–shoot ratio) were calculated. These data were analyzed by two-way analysis of variance to determine whether the nutrient treatments had any significant effect, whether the phenolic treatments had any significant effect, and whether there was a significant interaction between the two.

Results

The effects of coumaric and vanillic acids at different nutrient concentrations are complex, as can be appreciated upon examination of Fig. 1. Lower nutrient concentrations did clearly depress growth rate, as indicated by final dry weight. However, the phenolic compounds were uniformly inhibitory only at the lowest levels of nitrogen and phosphorus. At higher nutrient concentrations, the effects of phenolics were highly variable, and often enhanced the dry weight yield. In some instances the interaction between phenolics and nutrients was dramatic; for example, while 50 ppm vanillic acid had little effect on growth in the complete nutrient solution, at 1 ppm P this amount of phenolic caused a 38% reduction in growth ($t = 3.99$, $P < 0.01$).

Table 1 presents the results of four two-way analyses of variance of these data. Nutrient levels had a strong and significant effect on dry weight in all four subexperiments. Phenolics, because they had a negligible influence in some cases and because they had a highly variable influence in others, showed no significant treatment effects, although the effect of vanillic acid, when tested with various concentrations of nitrogen, was suggestive ($P = 0.06$). Significant interaction effects were shown by phosphorus and vanillic acid and by nitrogen and coumaric acid. This result indicates that vanillic acid did significantly influence growth, but this influence varied in strength and direction depending on the phosphorus supply; similarly, coumaric acid influenced growth in a manner which depended on the nitrogen supply.

Root–shoot ratios accurately reflected total dry weight, smaller plants possessing the higher root–shoot ratios. This generalization seemed to hold regardless of whether the small size was the result of nutrient deprivation or phenolic toxicity.

Discussion

Phenolic compounds at the concentrations used in this study did significantly influence growth, depending on the concentrations of nitrogen and phosphorus. Both total dry weight and the distribution of that dry weight were affected; under low nutrient concentrations, phenolics caused plants to be smaller and to have a larger proportion of their dry weight in roots than the control plants. This increase in the root–shoot ratio runs counter to the observation that phenolics sometimes depress root growth (Glass 1976; Schreiner and Reed 1908).

In this experiment the concentrations of nitrogen and phosphorus in the nutrient solution were critical in determining the toxicity of phenolic compounds. Only plants which were suffering somewhat from nutrient deprivation showed a significant and consistent inhibition by phenolics. There was no real indication of specificity in the interaction between phenolics and nutrients; either phenolic was inhibitory when either nutrient was limiting. Coumaric acid was approximately as inhibitory as vanillic acid, even though it was present at one fifth the concentration. These results tend to corroborate the ideas of Glass (1974) and Glass and Dunlop (1974) that phenolics have nonspecific effects on root cell membranes and therefore on ion uptake, and that the degree of inhibition of ion uptake caused by a phenolic compound is correlated with its lipid solubility and its ability to depolarize the membrane potentials of root cells. Of 12 naturally occurring phenolic compounds, vanillic acid was one of the least inhibitory to potassium uptake,

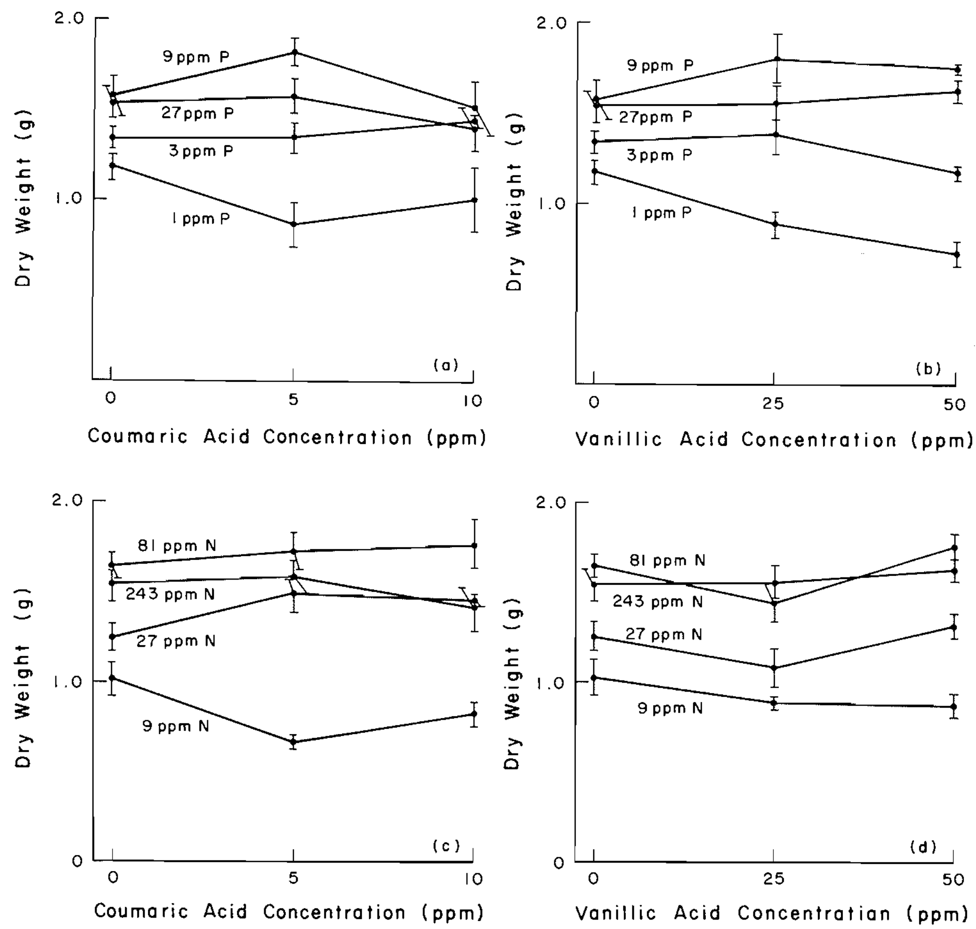

FIG. 1. Dry weight yield of barley plants at various levels of phosphorus and nitrogen, treated with several concentrations of coumaric and vanillic acids. Each point represents the sum of the above and belowground biomass per pot (three plants), averaged for four pots. The nitrogen concentration was maintained at 243 ppm while the phosphorus concentration varied, and the phosphorus concentration was maintained at 27 ppm while the nitrogen concentration varied.

TABLE 1. *F*-levels from four two-way analyses of variance showing the effects of different nutrient treatments, different phenolic treatments, and interactions

	F	Probability
Phosphorus and coumaric acid (Fig. 1*a*)		
Nutrient effect	15.627	<0.001
Phenolic effect	0.199	0.820
Nutrient–phenolic interaction	1.430	0.222
Phosphorus and vanillic acid (Fig. 1*b*)		
Nutrient effect	26.380	<0.001
Phenolic effect	0.844	0.436
Nutrient–phenolic interaction	2.559	0.032
Nitrogen and coumaric acid (Fig. 1*c*)		
Nutrient effect	41.344	<0.001
Phenolic effect	0.127	0.881
Nutrient–phenolic interaction	2.638	0.026
Nitrogen and vanillic acid (Fig. 1*d*)		
Nutrient effect	33.861	<0.001
Phenolic effect	2.974	0.060
Nutrient–phenolic interaction	0.609	0.722

while coumaric acid was intermediate; the most toxic phenolics were cinnamic and salicylic acids (Glass 1974).

The ecological significance of these results cannot be discussed without consideration of how barley compares with other species in its response to phenolic compounds, and how the concentrations of phenolics and nutrients used in this investigation compare with those of natural soils. Glass (1976) found that wheat, oat, ryegrass, barley grass (*Hordeum murinum* L.), and clover were all inhibited by phenolics in the same manner as barley, but that quackgrass (*Agropyron repens* L.), propagated from rhizomes, was resistant. Likewise Olmsted and Rice (1970) found species differences in resistance to phenolics among the successional species of Oklahoma old fields. Thus species and ecotypes certainly differ in their sensitivity to phenolic compounds.

As to the concentrations of these substances in

natural soils, Whitehead (1964) sampled British soils underlying four vegetation types and found the coumaric acid concentration to range from 1.48 to 6.90 ppm and the vanillic acid concentration to range from 1.18 to 8.24 ppm. Wang *et al.* (1967) investigated a number of agricultural soils in Taiwan and found somewhat lower concentrations: coumaric acid, 0–3.45 ppm, and vanillic acid, 0.08 ppm–0.82 ppm (accepting their estimate that the soils contained approximately 14% water). Thus the concentrations of vanillic acid which were used in this study, because they gave slight inhibition, were several to many times those of tested natural soils. Coumaric acid concentrations, on the other hand, followed the natural ones fairly closely.

It is very difficult to relate natural levels of nitrogen and phosphorus to those of this sand-culture experiment. Measurements of natural nutrient concentrations depend strongly on the type of extraction procedure used. Also, nutrients are continually renewed by natural soils, while they must diffuse to the root in sand culture. And finally, the activity of microorganisms in the rhizosphere may alter the nutrient concentrations perceived by plants in natural soils. Stout and Overstreet (1950) report that the phosphorus concentration in the soil solution is typically of the order of 1 ppm, yet using most extraction procedures, 10 ppm is considered deficient (Bingham 1966). Arable soils have nitrate concentrations ranging from 2 to 60 ppm of nitrogen as nitrate (Jones 1966). The lesser concentrations of nitrogen and phosphorus used in this experiment have been called "low" only in reference to the fact that they caused a substantial reduction in plant growth.

It has been suggested that allelopathy should be especially influential in arid regions, because bacterial decomposition and leaching of allelopathic agents in these areas would be relatively slow (Muller 1970; del Moral and Muller 1970). It now appears that allelopathy by means of phenolic compounds should also be most important in soils of low fertility, or during periods of the year of low fertility. Plants growing under nutrient stress often produce larger quantities of phenolics than plants with ample nutrients (Armstrong *et al.* 1970; Lehman and Rice 1972; del Moral 1972); and, as this investigation demonstrates, the inhibition of growth by phenolics at low concentrations may be significant only in association with nutrient deficiencies.

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